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DEVELOPMENT OF CELL LINES FROM THE SHEEP USED TO CONSTRUCT THE CHORI-243 OVINE BAC LIBRARY

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Two cell lines, designated MARC.OVSM, and MARC.OKF, were initiated from the aorta and kidney, respectively, obtained from the Texel ram used to make the CHORI-243 Ovine BAC library. These cell lines have been submitted to the NIA Aging Cell Repository at the Coriell Cell Repositories, Camden, NJ, USA, and will be made publicly available.

Keywords: Fibroblast; Karyotype; *Ovis aries*; Smooth muscle

INTRODUCTION

The CHORI-243 Ovine BAC library was constructed by Michael Nefedov in Pieter de Jong's laboratory at BACPAC Resources, Children's Hospital Oakland Research Institute (<http://bacpac.chori.org/library.php?=&id=162>), Oakland, CA, USA, following the cloning approach described by Osoegawa et al. (1). DNA used to develop the library was isolated from white blood cells of a Texel ram (animal #200118011) from the flock maintained at the U.S. Meat Animal Research Center (USMARC), Clay Center, NE, USA. Furthermore, DNA from this ram was used to generate the virtual sheep genome (<http://www.livestockgenomics.csiro.au/vsheep/>) and will likely be the template for generating the complete sheep genome sequence should funding become available. To provide a nearly unlimited source of DNA

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Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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to researchers performing genetic studies utilizing this BAC library, we developed cell lines from the aorta and kidney of this Texel ram.

MATERIALS AND METHODS

Experimental procedures were approved and performed in accordance with USMARC Animal Care Guidelines and the Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching (2). Texel ram #200118011 was humanely euthanized. A 2.5-cm³ section of aorta and a 3-cm³ section of kidney cortex were individually minced, and mechanically disrupted in 5 ml PBS using a polytron homogenizer. The homogenized tissue solutions were poured into sterile flasks containing 200 ml 0.25% trypsin in Hank's Balanced Salt Solution without calcium and magnesium (Gibco BRL, Grand Island, NY, USA) and stirred for 1 h at 37°C (3). The homogenates were then poured through a sterile tissue sieve (Collector[®], Bellco Glass, Inc., Vineland, NJ, USA) and centrifuged at 1000 X g, 4°C, for 10 min. The supernatants were decanted and replaced with Roswell Park Memorial Institute 1640 medium containing Antibiotic-Antimycotic (Gibco BRL) and 10% fetal bovine serum (FBS), and the cell pellets resuspended and centrifuged as described above. The supernatants were replaced with 40 ml fresh medium and the cells plated into two 75-cm² tissue culture flasks for each tissue type. The cultures were incubated at 37°C with 5% CO₂ in a humidified atmosphere overnight. The following day, the medium and non-adherent cells were removed from the flasks and each replaced with 20 ml fresh medium. To expand the cultures or to facilitate freezing, adherent cell monolayers were disrupted by treatment with Trypsin-EDTA (TE; Gibco BRL; 4). Cells were either placed back into culture or freezing medium for storage in LN₂ (5). For immunohistochemical analyses, cells were grown on 4-well culture slides, fixed with ice-cold absolute methanol, permeabilized with 0.1% triton X-100 in PBS, and blocked with 10% normal goat serum and 2% BSA in PBS. Primary antibodies used were rabbit polyclonal anti-von Willebrand factor/Factor VIII (Dako North America, Inc., Carpinteria, CA, USA), mouse monoclonal antibody anti- α -smooth muscle actin (anti- α -SMA; Sigma-Aldrich, St. Louis, MO, USA), mouse monoclonal (IgM) anti-fibroblast surface protein (anti-FSP; Sigma-Aldrich), and Cy3-conjugated mouse monoclonal anti-vimentin (Sigma-Aldrich). Secondary antibodies used were FITC-labeled goat anti-rabbit IgG (MP Biomedicals, Solon, OH, USA) and Rhodamine-labeled goat anti-mouse IgG (EMD Chemicals, Inc., San Diego, CA, USA) for detection of von Willebrand factor and α -SMA, respectively, and FITC-labeled anti-IgM (Sigma-Aldrich) for detection of FSP. Metaphase chromosome spreads were prepared for cytogenetic analysis by the commonly used "air-drying technique" (6), followed by G-banding. Metaphases were collected by colcemid treatment (10 ng/ml final concentration) for 45 min prior to harvest. The cells were removed by trypsin treatment, then swollen in hypotonic solution (Potassium Chloride 0.27%, Sodium Citrate Dihydrate 0.23%) for 15 min at 37°C, followed by fixation with cold Methanol:Acetic acid, 3:1. After three changes of fixative, drops of cell suspension were dropped onto clean wet slides and dried in a Thermotron environmental chamber set at 24°C and 44% relative humidity. The slides were allowed to dry thoroughly overnight and then treated at 90°C for 90 min to "age" them prior to banding by trypsin treatment and Wrights staining (7).

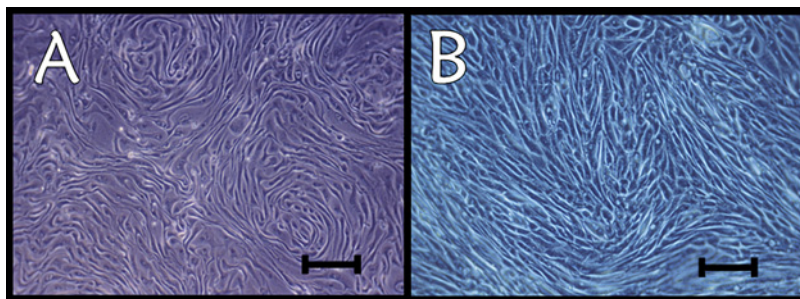


Figure 1 (A) MARC.OVSM and (B) MARC.OKF, phase contrast 25X magnification (bar = 0.2 μ).

Fifty metaphases from each culture were studied. Images were captured and karyotypes were prepared using the BandView[®] system (Applied Spectral Imaging, Inc., Vista, CA). The karyotypes were arranged according to ISCNDB 2000 (8). Karyotypic abnormalities were described using the terms and form specified by ISCN 2005 (9).

RESULTS AND DISCUSSION

Cell foci were evident in both cell cultures by day 7 and treated with TE to disperse the cells and stimulate formation of a monolayer. The kidney cells reached confluency 5 days later at about 7.5 million cells per 75-cm² flask and had the appearance of fibroblasts (Figure 1A). The cells obtained from the aorta reached confluency at about 1.75 million cells per 75-cm² flask 8 days after the initial foci

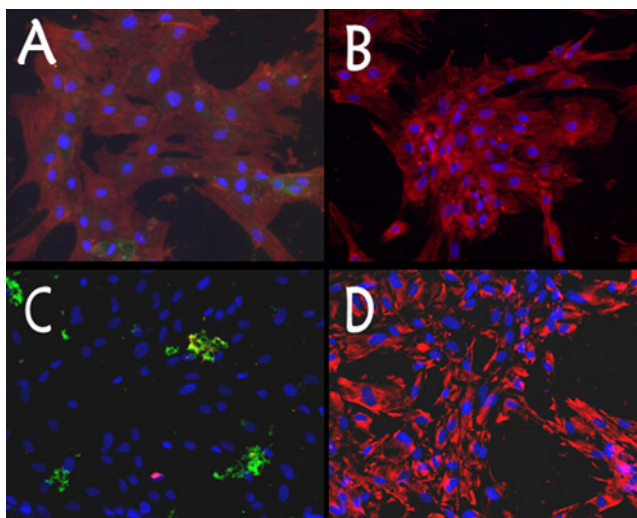


Figure 2 Immunohistochemical staining of MARC.OVSM ([A] α -SMA and [B] anti-vimentin) and MARC.OKF ([C] anti-FSP and [D] anti-vimentin).

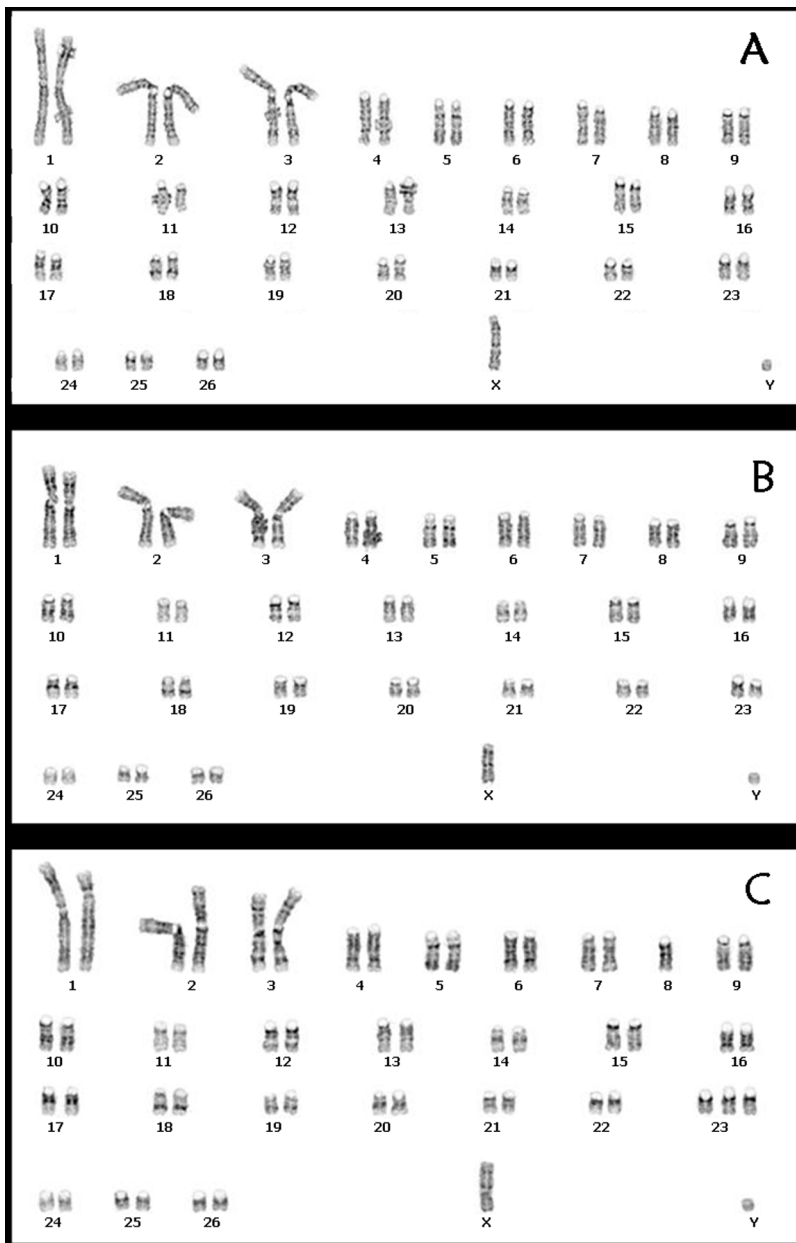


Figure 3 Representative karyotype of (A) MARK.OVSM, (B) MARK.OKF normal clone, and (C) MARK.OKF abnormal clone.

were dispersed and had the appearance of smooth muscle cells (Figure 1B). The cultures were maintained by subculturing at 1:5 (kidney cells) or at 1:10 (aortic cells) approximately once per week. Both cell lines were positive for vimentin staining

indicating the cells are of mesodermal origin (Figure 2), and negative for von Willebrand factor indicating the cell lines are not endothelial. The cells obtained from the aorta, but not the kidney, stained ubiquitously for α -SMA indicating they are smooth muscle. The staining pattern observed with anti-FSP when applied to the kidney cells was similar to that obtained in the same experiment with a bona fide human dermal fibroblast culture in which only a subset of the cells exhibited speckled staining (Figure 2). Cytogenetic analysis of the aorta smooth muscle cell culture demonstrated an apparently normal male *Ovis aries* karyotype 54,XY (Figure 3A). However, the kidney fibroblast culture is a mosaic that contains three clones: 53,XY,inv(1)(p31q11)-8,+23{15}/54,XY,inv(1)(p31q11)[8]/54,XY[27]. These abnormalities probably occurred *in vitro*, first the inversion 1, and then the loss of chromosome 8 and the gain of chromosome 23 from a cell carrying the inversion. The cell lines were designated MARC.OVSM (USMARC ovine vascular smooth muscle cells) and MARC.OKF (USMARC ovine kidney fibroblasts), and submitted to the NIA Aging Cell Repository at the Coriell Cell Repositories (<http://www.coriell.org/index.php/content/view/64/117/>). Following expansion, they will be available as AG19826 (MARC.OVSM) and AG19825 (MARC.OKF). At this time, MARC.OVSM has been continuously subcultured 38 times and MARC.OKF 35 times.

REFERENCES

1. Osoegawa K, Woon PY, Zhao B, Frengen E, Tateno M, Catanese JJ, de Jong PJ. An improved approach for construction of bacterial artificial chromosome libraries. *Genomics* 1998; 52(1):1–8.
2. FASS. Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching. 1st Ed., Federation of Animal Science Societies, Savoy, IL 1999.
3. Freshney RI. Culture of Animal Cells: A Manual of Basic Technique. 2nd Ed., Alan R. Liss, Inc., New York, 1987; 117.
4. Helgason CD. Culture of primary adherent cells and a continuously growing nonadherent cell line. In *Basic Cell Culture Protocols*; 3rd Ed., Helgason CD and Miller CL, Eds; Humana Press, New Jersey, 2005; 8.
5. Yokoyama WM. Current protocols in immunology. Coligan JE, Kruisbeek AM, Margulies DH, Shevach EM, and Strober W., Eds; John Wiley & Sons, Inc., New Jersey, 1997; A.3G.1–A.3G.3.
6. Rothfels KH, Siminovitch L. An air-drying technique for flattening chromosomes in mammalian cells grown in vitro. *Stain Technol* 1958; 33(2):73–77.
7. Francke U, Oliver N. Quantitative analysis of high-resolution trypsin-giemsa bands on human prometaphase chromosomes. *Hum Genet* 1978; 45(2):137–165.
8. Cribiu EP, Di Berardino D, Di Meo GP, Eggen A, Gallagher DS, Gustavsson I, Hayes H, Iannuzzi L, Popescu CP, Rubes J, Schmutz S, Stranzinger G, Vaiman A, Womack J. International system for chromosome nomenclature of domestic bovids (ISCNDB 2000). *Cytogenet Cell Genet* 2001; 92(3–4):283–299.
9. ISCN 2005: An international system for human cytogenetic nomenclature (2005). In L.G. Shaffer, N. Tommerup, and Karger S. Basel, Eds; (2005).